

**GOMEZ-REY, M.X.<sup>1\*</sup>; GARCIA-MARCO, S.<sup>1</sup>; GONZÁLEZ-PRIETO, S.J.<sup>1</sup> (2014). Soil P and cation availability and crop uptake in a forage rotation under conventional and reduced tillage. *Soil Use and Management* 30, 445-453.**

<sup>1</sup> Instituto de Investigaciones Agrobiológicas de Galicia, IIAG-CSIC. Apartado 122. E-15780 Santiago de Compostela, Spain

\* Corresponding author: mxgomez@iiag.csic.es

## **Abstract**

Long-term conservation tillage can modify vertical distribution of nutrients in soil profiles and alter nutrient availability and yields of crops. This study aimed to evaluate the effect of 14 years of conventional (CT) and reduced tillage (RT) on soil macronutrient availability (0-5, 5-15, 15-30 cm) and uptake by Italian ryegrass and maize in a forage rotation under a temperate-humid climate (NW Spain). Soil contents of total C, plant available Ca, Mg, Na, K and P and their uptake by plants were evaluated over two years. The three-way ANOVA showed that tillage and its interactions with soil depth and sampling date have little influence on soil C and macronutrients contents (< 13% of variance explained). In the topsoil layer, all studied variables (except K) increased in RT compared with CT, but they remained unchanged (C, Ca and Na) or decreased (Mg, K and P) in deeper layers. Crop yields were greater with RT than CT during the year with soil water deficit periods, while limited tillage effect was found in the other year. Whereas no differences were obtained for maize, nutrient concentration (Mg, Na, K and P) in ryegrass increased under RT. Conservation tillage improved surface soil fertility, maize yield and ryegrass nutrient content.

**Keywords:** bent-leg subsoiler; fodder maize; temperate-humid; ploughing tillage; reduced tillage.

## **Introduction**

Conservation tillage decreases soil disturbance caused by conventional ploughing and aims to reduce soil erosion and degradation (Holland, 2004). Usually, any soil disturbance is confined to a shallow working depth and there is no soil inversion (no-tillage or reduced tillage) to maintain crop residues on the soil surface (Peigne, 2007). Reduced tillage minimises the integration of soil and crop residues, slows the decomposition of the latter (Balesdent et al. 2000) and leads to SOM stratification, with increased surface concentrations (Ogle et al. 2005; Martín-Rueda et al. 2007; Thomas et al. 2007).

Superficial accumulation of crop residues and surface application of fertilizer may modify the vertical distribution of nutrients, leading to greater concentrations in the top layer (Edwards et al. 1992; Franzluebbers & Hons, 1996; Martín-Rueda et al. 2007). These changes in the spatial distribution of nutrients can modify their availability over time and total quantity, with possible consequences for species relationships, including competition between crops (Franzluebbers & Hons, 1996), and

nutrient deficiencies by altering fertilizer requirements and placement (Holanda et al. 1998). Unfortunately, responses by crops to changes in nutrient availability have not been fully examined (Soane et al. 2012). Because of improved nutrient concentration in soil surface, it might be expected that nutrient content in crops growing under reduced tillage should also be greater; however, contradictory results have been observed (Martín-Rueda et al. 2007; Krauss et al. 2010; Martínez et al. 2013) likely due to differences in soil, climate and crops.

The effect of conservation tillage on yields depends on species, climate and soil texture (Martínez et al. 2013). In humid and sub-humid regions, the conservation systems can cause yield losses in wet seasons and inconsistent effects in dry seasons (Soane et al. 2012). In contrast, by increasing infiltration and reducing runoff and evaporation, conservation tillage can increase soil water availability and mitigate mid-season drought effects (Thierfelder & Wall, 2009), resulting in larger yields in water-limited environments (Moreno et al. 2011; Soane et al. 2012). Adoption of new practices involves changes in management that can

temporarily reduce crop yields, so long-term field experiments are required to properly evaluate the impacts of conservation tillage.

Conservation tillage has been mainly adopted in the Americas, Australia and South Africa but there is scepticism about its suitability in European soil, climate and cropping conditions (Stagnari et al. 2010). In Europe, detailed information on the environmental benefits of conservation tillage is scarce and heterogeneous (Holland, 2004). In semiarid areas of Southern Europe no-tillage is effective for reducing soil erosion, improving water supply to plants and enhancing crop yield (e.g. De Vita et al. 2007; Moreno et al. 2011; Thierfelder & Wall, 2009). In temperate areas, conservation tillage may have substantial environmental and economical advantages (Gruber et al. 2012; Soane et al. 2012). In Spain, forage maize cover about 89,000 ha, 64% of which in the north-western temperate-humid zone, where it is the most common crop under conservation tillage, which has economic and timeliness advantages without adversely affecting yields (Bueno et al. 2007) and improving topsoil physical (Bueno et al. 2006), chemical (García-Marco

et al. 2014; Gómez-Rey et al. 2012) and biological properties (Díaz-Raviña et al. 2005).

We hypothesized that reduced tillage would lead to nutrient stratification in soil, which in turn would affect yield and nutrient contents of crops. This study aimed to evaluate the long-term effect (14 years) of conventional and reduced tillage on crop yields, plant nutrient contents, and soil C and nutrient distribution in a ryegrass-maize rotation.

## Material and methods

### Site description

The experimental field was located in the Gayoso-Castro farm (NW Spain, 43°06'N, 7°27' W, 420 m a.s.l.). At As Rozas, Rubiás and Lugo meteorological stations, placed within 17 km from the farm and at similar altitudes, during the study period (October 2006-October 2008) the annual temperature was  $12.1 \pm 0.7$  °C (mean  $\pm$  s.d) and mean annual rainfall was 941 mm (coefficient of variation: 19%); rainfall mainly occurred in the October to June period (Fig. 1; Meteogalicia, 2013).

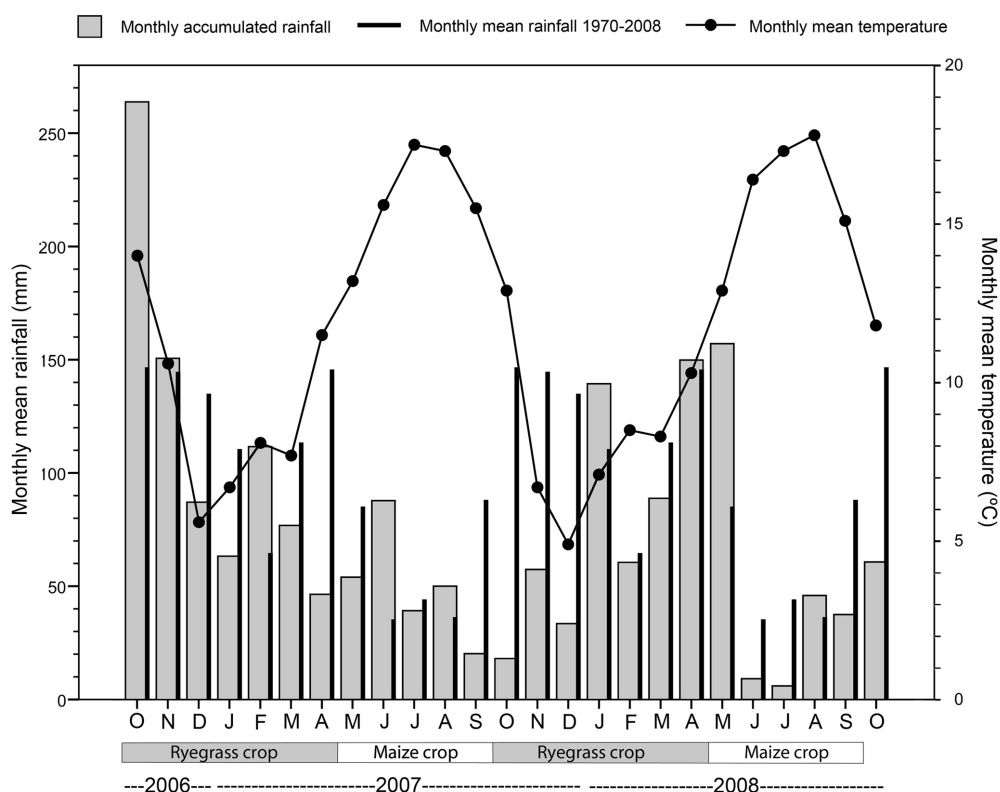


Fig. 1. Monthly mean temperature (°C, points connected by a line) and monthly rainfall (mm, wide grey bars) during the growing season of ryegrass and maize. For comparison, the vertical dark lines show the monthly mean precipitation during the 1970-2008 period.

The soil is a Phaeozem Gleyic (IUSS Working Group, 2006) developed over sandy-clayey deposits, with a sandy loam topsoil (70 % of sand in the 0-5 cm) and acidic pH<sub>H2O</sub> (about 5.5).

Prior to the experiment there were no significant differences between the plots assigned to the different tillage systems for the variables studied, which shows acceptable spatial homogeneity of the experimental field. Since 1994, a rotation of forage maize (*Zea mays* L.) and Italian ryegrass (*Lolium multiflorum* L.) has been annually cultivated in two adjacent areas with different management: conventional plough tillage (CT) and reduced tillage (RT). Each area was divided in nine replicate plots (4 m x 3 m, with 1-m wide buffer zones between them). Maize was sown in rows 0.75 m apart (95,000 plants/ha) in late May and harvested in late September. Ryegrass was sown in rows 0.17 m apart (40 kg/ha) in late October and harvested in early May. In RT, before maize sowing, the adventitious vegetation was eliminated adding glyphosate (36% of active ingredient, 5 L/ha). Under RT, after 8-yr of no-till drilling on stubble of the preceding crop, the management was changed to reduced tillage and the soil was annually loosened with a bent-leg subsoiler to a depth of 30 cm before maize seeding aiming to revert the increasing soil compaction and decreasing emergence of maize seedlings. Under CT, the soil was ploughed at 25-30 cm with a reversible plough in May and October to incorporate crop residues and to prepare a seedbed. Further agrochemical treatments were similar for both tillage systems. For the maize crops, plots were treated with herbicides (33% acetachlor and 16.5% atrazine, 4 L/ha), insecticide (48% clorpiriphos, 0.33 L/ha) and fertilizer (N: 63 kg/ha; P: 55 kg/ha; K: 157 kg/ha), which was applied in sowing at 10 cm of depth. During the ryegrass cultivation, plots were surface fertilized in early October (N: 27 kg/ha; P: 23 kg/ha; K: 67 kg/ha) and early March (N as NH<sub>4</sub>NO<sub>3</sub>: 81 kg/ha).

#### *Soil and plant sampling*

Soil was sampled at the beginning of the experiment (October 2006) and just after ryegrass (May 2007 and 2008) and maize (October 2007 and 2008) harvest. In each plot, soil was sampled (0-5, 5-15 and 15-30 cm depth) with a stainless steel probe (4 cm internal diameter) at 8 points uniformly distributed between the rows; afterwards it was thoroughly mixed into a composite sample per plot,

sieved (< 2 mm) and air-dried. Soil water-holding capacity (WHC) was determined in a Richards membrane-plate extractor (10 kPA pressure) and soil texture by the international mechanical analysis method. In these plots, García-Marco et al. (2014) found that texture and WHC did not vary among sampling dates and that neither tillage nor depth have significant effects on texture (Table 1); however, both factors affected the WHC, their strong interaction (63% of variance explained) showing that WHC decreased significantly with depth only in RT. Crops were harvested in May (rye-grass) and October (maize) of 2007 and 2008. In each plot, all plants were cut at the base and weighed for determining yields. For chemical analyses plants from the plot centre (75 cm inward from the edge) were collected separately, homogenized and crushed (< 4 cm) *in situ*, and a subsample was dried at 60 °C for 10 h and crushed again (< 4 mm).

#### *Chemical analyses*

The dry matter content of soils and plants was assessed by oven-drying subsamples at 110 °C to constant weight. Soil total C was measured on finely ground samples (< 100 µm) with an elemental analyser (Carlo Erba CNS 1508). For available nutrients analyses, soil (10 g) was shaken for 2 h with a solution of 1 M NH<sub>4</sub>Ac and 0.005 M DTPA; the extracts were filtered and analysed for Ca, Mg, Na, K and P by simultaneous ICP-OES (Varian Vista Pro, Mulgrave, Australia).

To determine the total nutrient content, plant materials were finely ground (< 100 µm) and subsamples (500 mg) were digested for 55 min with 8 mL of 65% HNO<sub>3</sub> and 25 mL of 30% H<sub>2</sub>O<sub>2</sub> in Teflon containers in a microwave digestion unit (Milestone 1200 Mega, Sorisole, Italy). Once cooled, the solutions were filtered, transferred to 25 mL volumetric flasks and made to volume with water. The total Ca, Mg, Na, K and P contents were measured by simultaneous ICP-OES. Analytical-grade chemicals were obtained from Merck Chemical Co., quantitative cellulose filter paper from Filter-laboratory and solutions were prepared with type I water.

All analyses were carried out in duplicate and the mean of both analyses was used in the statistical procedure.

Table 1. Particle size and water holding capacity (WHC) of the soil (< 2 mm) collected under conventional (CT) or minimum (MT) tillage; values are mean  $\pm$  standard deviation for the four sampling dates.

	CT			MT		
	0-5 cm	5-15 cm	15-30 cm	0-5 cm	5-15 cm	15-30 cm
Sand (g kg <sup>-1</sup> dw)	69 $\pm$ 3 aA	66 $\pm$ 4 aA	72 $\pm$ 6 aA	71 $\pm$ 1 aA	72 $\pm$ 5 aA	71 $\pm$ 2 aA
Silt (g kg <sup>-1</sup> dw)	13 $\pm$ 1 aA	16 $\pm$ 2 aA	12 $\pm$ 6 aA	13 $\pm$ 1 aA	10 $\pm$ 8 aA	13 $\pm$ 1 aA
Clay (g kg <sup>-1</sup> dw)	17 $\pm$ 2 aA	19 $\pm$ 3 aA	16 $\pm$ 1 aA	16 $\pm$ 1 aA	19 $\pm$ 3 aA	16 $\pm$ 3 aA
WHC (g H <sub>2</sub> O kg <sup>-1</sup> dw)	315 $\pm$ 16 bA	318 $\pm$ 22 bA	306 $\pm$ 3 aA	392 $\pm$ 17 aA	358 $\pm$ 2 aB	310 $\pm$ 12 aC
WHC (Mg H <sub>2</sub> O ha <sup>-1</sup> )	83 $\pm$ 4 b	169 $\pm$ 12 b	243 $\pm$ 2 a	104 $\pm$ 5 a	190 $\pm$ 1 a	246 $\pm$ 10 a

Different lowercase letters indicate significant differences ( $p < 0.05$ ) between tillage systems for the same soil depth. Different uppercase letters indicate significant differences ( $p < 0.05$ ) among depths for the same tillage system; due to the different thickness of soil layers, this comparison was not done for WHC expressed as Mg H<sub>2</sub>O ha<sup>-1</sup>.

### Calculation and statistical analysis

Soil data were statistically analysed by three-way ANOVA (with tillage system, soil depth and sampling date as factors) and those of plant variables by two-way ANOVA (with tillage and sampling as factors). After checking the equality of variances among groups with Levene's test, significant differences among their means were established at  $p < 0.05$  using the Bonferroni's test for multiple comparisons. With unequal variances, the original data were subjected to the Tukey's ladder of power, or to Cox-Box transformations to obtain homoscedasticity and then significant differences among groups were established as previously explained. The proportion of the variation accounted for each factor or interaction in the ANOVA was determined by the partial eta-squared ( $\eta_p^2$ ) statistic. Statistical procedures were performed using SPSS 15.0.

## Results

### Soil C and macronutrients

Soil total-C was affected by the tillage system (7.2% of variance explained, Table 2), average concentrations being higher under RT than CT. However, the tillage  $\times$  depth (TxD) interaction showed that the increase was only significant in the top layer. Depth explained 34% of the variance of soil C, which was more stratified under RT than under CT.

Overall, no significant effect was observed of

tillage system or soil depth on available-Ca (Table 2).

Although globally the available-Mg was unaffected by the tillage system, the TxS interaction showed that was greater for RT than for CT in the topsoil (0-5 cm) and smaller in the deepest layer (15-30 cm). These differences depend on sampling date (significant TxS interaction) and were only significant in half of samplings. About 47% of the variance was explained by soil depth and, in both tillage systems, Mg concentrations were greater in the 0-5 cm layer than in the others.

The three-way ANOVA showed a small effect of tillage system (2.5% of variance explained) on available-Na, values being greater under RT than CT, although the difference was only significant in the 0-5 cm soil layer (Table 2).

Available-K was greater under CT than RT in the 5-15 and 15-30 cm layers but similar in the topsoil (Table 2). Soil depth strongly affects K levels (70% of variance explained), which were more stratified under RT than CT.

Available-P was slightly affected by the tillage system (3.4% of variance explained, Table 2), average values being significantly greater under CT than RT. However, the three-way interaction showed that, for half the dates, RT had significantly more P than CT in the topsoil but less in the deeper layers. In both tillage systems, available-P decreased with soil depth (which explains half of the variance), although the stratification was greater under RT than CT (Table 2).

**Table 2** Mean values  $\pm$  standard deviation of soil total C (g kg<sup>-1</sup>) and available Ca, Mg, Na, K, and P (mg kg<sup>-1</sup>) and results of the three-way ANOVA with tillage system (CT: conventional tillage, MT: minimum tillage), soil depth and sampling date as factors.

	Total C	Available Ca	Available Mg	Available Na	Available K	Available P
Tillage						
CT	50.4 $\pm$ 5.7 <sup>b</sup>	692 $\pm$ 238 <sup>a</sup>	35.1 $\pm$ 12.8 <sup>a</sup>	28.4 $\pm$ 9.5 <sup>b</sup>	94.6 $\pm$ 38.6 <sup>a</sup>	6.6 $\pm$ 3.1 <sup>a</sup>
MT	53.7 $\pm$ 8.5 <sup>a</sup>	674 $\pm$ 162 <sup>a</sup>	37.4 $\pm$ 18.2 <sup>a</sup>	30.7 $\pm$ 13.8 <sup>a</sup>	93.5 $\pm$ 60.5 <sup>b</sup>	6.1 $\pm$ 2.8 <sup>b</sup>
Depth						
0-5 cm	57.4 $\pm$ 8.1 <sup>a</sup>	663 $\pm$ 197 <sup>a</sup>	48.9 $\pm$ 18.4 <sup>a</sup>	33.8 $\pm$ 9.7 <sup>a</sup>	141.4 $\pm$ 59.5 <sup>a</sup>	8.5 $\pm$ 3.1 <sup>a</sup>
5-15 cm	50.9 $\pm$ 4.7 <sup>b</sup>	690 $\pm$ 223 <sup>a</sup>	30.5 $\pm$ 10.2 <sup>b</sup>	26.6 $\pm$ 13.0 <sup>b</sup>	75.7 $\pm$ 22.6 <sup>b</sup>	6.0 $\pm$ 2.3 <sup>b</sup>
15-30 cm	47.9 $\pm$ 5.4 <sup>c</sup>	695 $\pm$ 188 <sup>a</sup>	29.1 $\pm$ 7.4 <sup>b</sup>	28.3 $\pm$ 11.7 <sup>b</sup>	65.6 $\pm$ 14.7 <sup>c</sup>	4.5 $\pm$ 1.9 <sup>c</sup>
Date						
May-2007	52.1 $\pm$ 7.2 <sup>a</sup>	823 $\pm$ 245 <sup>a</sup>	46.1 $\pm$ 14.5 <sup>a</sup>	22.8 $\pm$ 7.6 <sup>c</sup>	90.2 $\pm$ 31.0 <sup>b</sup>	8.4 $\pm$ 3.2 <sup>a</sup>
October-2007	52.1 $\pm$ 8.0 <sup>a</sup>	715 $\pm$ 167 <sup>ab</sup>	38.9 $\pm$ 18.2 <sup>b</sup>	23.7 $\pm$ 7.9 <sup>c</sup>	125.9 $\pm$ 76.3 <sup>a</sup>	6.9 $\pm$ 2.8 <sup>b</sup>
May-2008	52.0 $\pm$ 7.2 <sup>a</sup>	561 $\pm$ 148 <sup>c</sup>	29.8 $\pm$ 10.6 <sup>c</sup>	26.3 $\pm$ 5.7 <sup>b</sup>	74.2 $\pm$ 19.9 <sup>c</sup>	6.1 $\pm$ 2.3 <sup>b</sup>
October-2008	52.1 $\pm$ 7.2 <sup>a</sup>	637 $\pm$ 136 <sup>b</sup>	30.2 $\pm$ 12.7 <sup>c</sup>	45.6 $\pm$ 8.5 <sup>a</sup>	83.3 $\pm$ 35.4 <sup>c</sup>	4.1 $\pm$ 1.6 <sup>c</sup>
Tillage x Depth						
CTx0-5 cm	52.8 $\pm$ 6.1 <sup>bA</sup>	630 $\pm$ 205 <sup>bA</sup>	42.6 $\pm$ 15.2 <sup>bA</sup>	30.1 $\pm$ 7.2 <sup>bA</sup>	131.2 $\pm$ 42.2 <sup>aA</sup>	8.0 $\pm$ 3.6 <sup>bA</sup>
CTx5-15 cm	51.0 $\pm$ 4.4 <sup>aA</sup>	706 $\pm$ 273 <sup>aAB</sup>	31.7 $\pm$ 11.0 <sup>aB</sup>	25.9 $\pm$ 11.6 <sup>aB</sup>	78.4 $\pm$ 21.3 <sup>aB</sup>	6.7 $\pm$ 2.6 <sup>aB</sup>
CTx15-30 cm	47.4 $\pm$ 5.0 <sup>aB</sup>	741 $\pm$ 222 <sup>aB</sup>	30.6 $\pm$ 7.2 <sup>aB</sup>	29.1 $\pm$ 8.9 <sup>aA</sup>	72.9 $\pm$ 11.9 <sup>aB</sup>	5.1 $\pm$ 2.2 <sup>aC</sup>
MTx0-5 cm	62.0 $\pm$ 7.3 <sup>aA</sup>	696 $\pm$ 185 <sup>aA</sup>	55.2 $\pm$ 19.2 <sup>aA</sup>	37.5 $\pm$ 10.6 <sup>aA</sup>	152.9 $\pm$ 73.4 <sup>aA</sup>	9.1 $\pm$ 2.4 <sup>aA</sup>
MTx5-15 cm	50.8 $\pm$ 5.0 <sup>aB</sup>	676 $\pm$ 163 <sup>aA</sup>	29.2 $\pm$ 9.1 <sup>aB</sup>	27.3 $\pm$ 14.5 <sup>aB</sup>	72.9 $\pm$ 23.8 <sup>bB</sup>	5.4 $\pm$ 1.7 <sup>bB</sup>
MTx15-30 cm	48.2 $\pm$ 5.7 <sup>aB</sup>	651 $\pm$ 137 <sup>aA</sup>	27.5 $\pm$ 7.3 <sup>bB</sup>	27.6 $\pm$ 13.9 <sup>aB</sup>	58.0 $\pm$ 13.5 <sup>bC</sup>	3.8 $\pm$ 1.0 <sup>bC</sup>
$\eta^2$ Tillage	0.072 ***	0.000 n.s.	0.000 n.s.	0.025 *	0.065 ***	0.034 *
$\eta^2$ Depth	0.342 ***	0.013 n.s.	0.472 ***	0.292 ***	0.708 ***	0.542 ***
$\eta^2$ Date	0.000 n.s.	0.267 ***	0.410 ***	0.738 ***	0.366 ***	0.519 ***
$\eta^2$ Tillage x Depth	0.117 ***	0.036 *	0.112 ***	0.116 ***	0.102 ***	0.142 ***
$\eta^2$ Tillage x Date	0.017 n.s.	0.011 n.s.	0.070 **	0.130 ***	0.028 n.s.	0.102 ***
$\eta^2$ Depth x Date	0.057 n.s.	0.065 *	0.152 ***	0.251 ***	0.238 ***	0.083 *
$\eta^2$ Tillage x Depth x Date	0.047 n.s.	0.035 n.s.	0.041 n.s.	0.034 n.s.	0.093 ***	0.036 n.s.

For the Tillage, Depth and Date factors, different lowercase letters in the same column indicate significant differences ( $p < 0.05$ ). For the tillage x depth interaction, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depths for the same tillage system. Partial  $\eta^2$ : proportion of the variation accounted for each factor or interaction in the ANOVA.

ns: not significant. \* Significant at  $p < 0.05$ . \*\* Significant at  $p < 0.01$ . \*\*\* Significant at  $p < 0.001$ .

## Plant nutrients

In 2007, maize (CT: 6613 kg/ha; RT: 6641 kg/ha) and ryegrass (3158 and 3114 kg/ha) yields were unaffected by tillage. However, in 2008 both crops yielded more in RT (ryegrass 4903 kg/ha; maize 7313 kg/ha) than in CT (4425 and 4755 kg/ha, respectively).

For ryegrass, a significant effect of date on all nutrient concentrations was observed, average values being higher in 2007 than in 2008 (Table 3). A similar trend was observed for Na and K in maize, but Mg and P levels were higher in 2008 than in

2007. Calcium concentrations in ryegrass were unaffected by the tillage system (Table 3, Fig. 2), which had a significant influence on Mg, Na, K and P (17%, 59%, 19% and 36% of variance explained, respectively), values being higher under RT than CT. However, these differences depended on sampling and were only significant in 2007 for Mg and 2008 for P and K (Fig. 2). No tillage effect was observed for nutrient concentration in maize, but the TxS interaction showed a K reduction in 2007 under RT, when compared with CT.

Table 3. Results of the two-way ANOVA for the concentration (mg kg<sup>-1</sup>) and uptake (kg ha<sup>-1</sup>) of Ca, Mg, Na, K and P in the aboveground biomass of ryegrass and maize with date (may-2007 and may-2008 for ryegrass; oct-2007 and oct-2008 for maize) and tillage system (conventional plough tillage, minimum tillage with yearly subsoiling) as factors.

			Date		Tillage		Date x Tillage	
			partial $\eta^2$	p	partial $\eta^2$	p	partial $\eta^2$	p
Ca	concentration	ryegrass	0.617	***	0.006	ns	0.139	*
		maize	0.017	ns	0.010	ns	0.002	ns
	uptake	ryegrass	0.102	ns	0.019	ns	0.004	ns
		maize	0.025	ns	0.075	*	0.048	ns
Mg	concentration	ryegrass	0.534	***	0.171	*	0.010	ns
		maize	0.275	***	0.017	ns	0.081	ns
	uptake	ryegrass	0.029	ns	0.168	*	0.021	ns
		maize	0.001	ns	0.111	*	0.111	ns
Na	concentration	ryegrass	0.273	**	0.585	***	0.043	ns
		maize	0.184	*	0.003	ns	0.087	ns
	uptake	ryegrass	0.023	ns	0.518	***	0.077	ns
		maize	0.130	*	0.024	ns	0.027	ns
K	concentration	ryegrass	0.349	***	0.192	**	0.080	ns
		maize	0.682	***	0.072	ns	0.134	*
	uptake	ryegrass	0.212	**	0.298	***	0.200	**
		maize	0.108	ns	0.008	ns	0.177	*
P	concentration	ryegrass	0.497	***	0.356	***	0.060	ns
		maize	0.560	***	0.032	ns	0.070	ns
	uptake	ryegrass	0.374	***	0.353	***	0.223	**
		maize	0.049	ns	0.131	*	0.144	0

<sup>a</sup>Partial  $\eta^2$ : proportion of the variation accounted for each factor or interaction in the ANOVA.  
ns: not significant. \* Significant at  $p < 0.05$ . \*\* Significant at  $p < 0.01$ . \*\*\* Significant at  $p < 0.001$ .

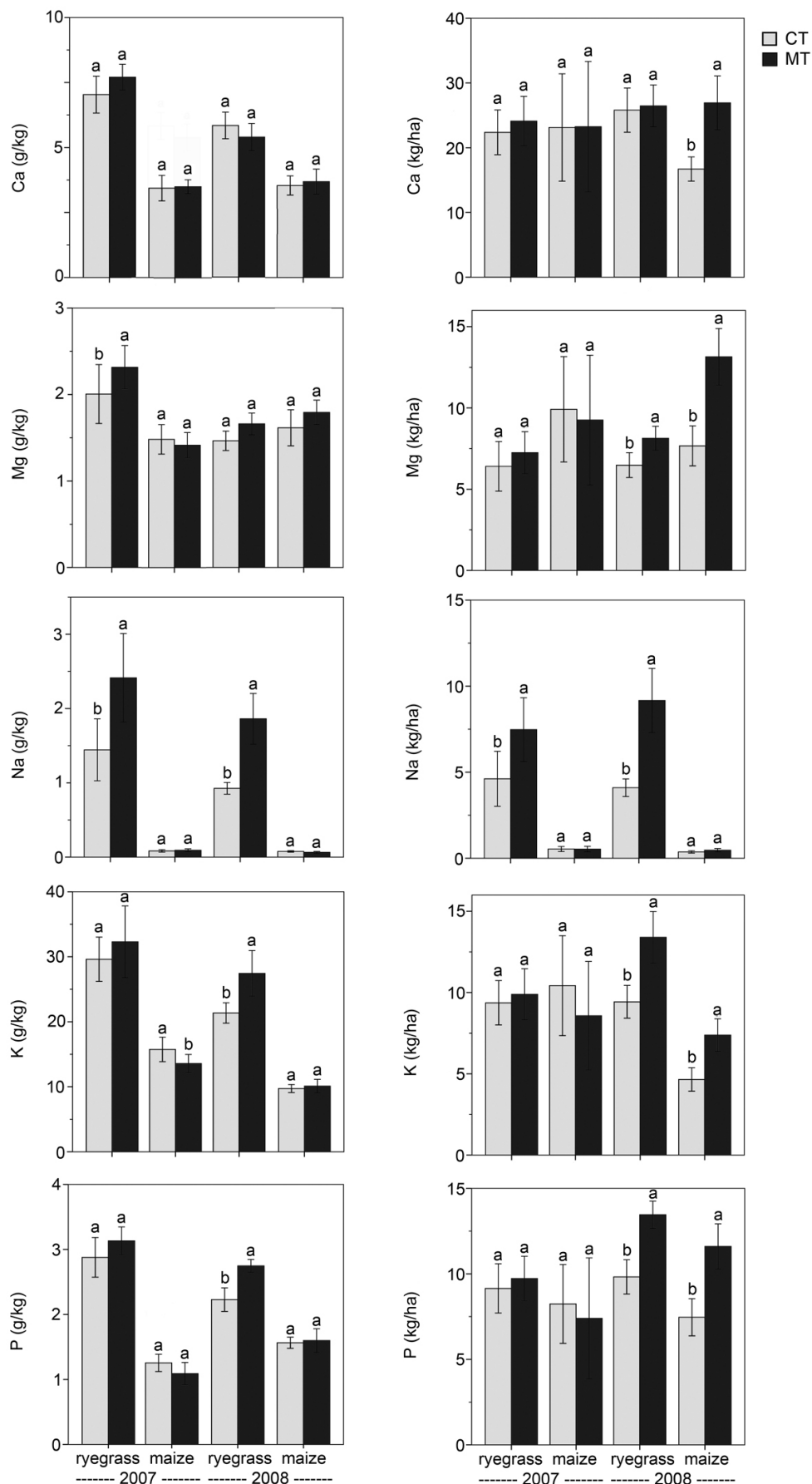


Fig. 2. Plant concentration and uptake (g/kg) of Ca, Mg, Na, K and P for ryegrass and maize growing under conventional plough tillage (CT) or reduced tillage with yearly subsoiling (RT). For each growing season and a given crop, different letters indicate differences between tillage systems ( $n = 9$ ;  $p < 0.05$ ). Bars represent  $\pm$  standard deviation.

Based on the average of both years, the amount of Mg, Na, K and P taken up by ryegrass was greater in RT than CT, a similar result being found for Ca, Mg and P in maize (Table 3). In 2008 the differences were statistically significant while in 2007 this was only true for ryegrass Na (Fig. 2). Moreover, in 2008 larger crop outputs of K were observed for maize in RT than CT.

## Discussion

After 14 years, little influence of tillage system (< 7% of variance explained) or TxD and TxS interactions (<14% of variance explained) on soil C and cations was found. The results support our hypothesis that RT increases nutrient stratification, as nutrients increased significantly in the topsoil (C, Ca, K and P by 10-17%; Na and Mg by 24-29%) but they remained unchanged or decreased in deeper layers. Moreover, the results confirmed partially the hypothesized change in crops yields (only the year with summer drought) and nutrient contents (only for ryegrass).

Conservation tillage led to soil C accumulation in the upper layer, consistent with reports from long-term studies in temperate-humid (Ogle et al. 2005; Gómez-Rey et al. 2012) and semi-arid regions (Moreno et al. 2011). This increase is attributable to crop residues being kept on the surface and less soil disturbance with conservation tillage, which reduce SOM decomposition (Balesdent et al. 2000), and to differences in C inputs. Greater yields were obtained under conservation tillage and, therefore, more plant residues were left on the field. Although changes in total C declined with depth, a significant accumulation of 3.0 Mg C/ha was observed for the 0-30 cm layer under RT compared with CT. This accumulation rate (211 kg C/ha/yr) was smaller than the 330-570 kg/ha/yr after conversion of conventional to no-tillage (Puget & Lal 2005; West & Post 2002). Our smaller C accumulation rate under RT may be explained by the large outputs associated with forage removal and no addition of organic amendments, as also reported Gadermaier et al. (2012).

The lack of tillage effect for soil available-Ca is consistent with the findings for calcareous soils (Franzluebbers & Hons, 1996; Thomas et al. 2007; Wright et al. 2007), where the high Ca stock minimized the tillage impact, but contrast with the surface reduction (Houx et al. 2011) or increase

(Edwards et al. 1992) also reported for no-tillage. These contrasting results are probably related to the low OM inputs in our study, as increased extractable Ca levels have been related to the higher SOM contents under no-tillage (Edwards et al. 1992). While available-Ca increases with proximity to bedrock in calcareous soils (Wright et al. 2007), in our soils it was unaffected by depth. The similar pattern to soil pH ( $r = 0.462$  with  $pH_{H_2O}$  and  $0.501$  with  $pH_{KCl}$ ;  $p < 0.01$ ;  $n = 213$ ) suggested that available-Ca could be influenced by liming, as recorded Guzman et al. (2006).

Considering the correlation between total-C and available-Mg ( $r = 0.535$ ,  $p < 0.01$ ;  $n = 213$ ), the higher Mg content in the topsoil layer under RT may be related to SOM accumulation, as reported Edwards et al. (1992) for no-tillage. A similar effect was observed in the top 2.5 cm under no-tillage (Guzman et al. (2006), although Thomas et al. (2007) reported Mg depletion in the 0-10 cm depth under reduced and no-tillage, a result attributed to changes in pH due to SOM accumulation. Tillage effects on Mg availability in sub-surface soil layers have been scarcely evaluated but similar (Franzluebbers & Hons, 1996; Wright et al. 2007) or smaller (Guzman et al. 2006) contents have been reported for the plough layer of conservation tillage. Although Guzman et al. (2006) related the Mg depletion in subsurface layers to changes in soil  $pH_{H_2O}$ , we found no significant correlations and the smaller Mg contents in the 15-30 cm layer of RT should be related to plant uptake, as more Mg was removed under RT.

Contrasting with the lack of effect (Franzluebbers & Hons, 1996; Houx et al. 2011) or the lower exchangeable-Na (Thomas et al. 2007) reported for reduced or no-tillage, we found slightly higher Na contents in the topsoil layer under RT, possibly due to reduced leaching losses. In both years, differences between tillage systems were wider in October than in May probably due to a greater Na removal by ryegrass than by maize, and also to leaching during the rainy autumn and winter.

Despite the surface K accumulation usually observed under conservation tillage (Franzluebbers & Hons, 1996; Guzman et al. 2006; Martín-Rueda et al. 2007; Thomas et al. 2007; Houx et al. 2011), we found no effect likely due to: a) the high mobility of K, readily leachable in rainy regions (Edwards et al. 1992); and b) the forage crops remove most of the aboveground biomass, leading to relatively low OM



inputs to soil. The depletion of K in deeper layers under RT was similar to the reported by Houx et al. (2011) after 18 years of no-tillage in the 5-10 cm layer and should be related to crop uptake that was higher during 2008 under RT.

As usually reported for conservation tillage (Edwards et al. 1992; Franzluebbers & Hons, 1996; Martín-Rueda et al. 2007; Houx et al. 2011; Gadermaier et al. 2012), RT led to higher available-P in the upper soil layer. This result is due to fertilization (Houx et al. 2011) and decomposition of crop residues (Franzluebbers & Hons, 1996; Wright et al. 2007) at the soil surface. Whatever the tillage system, available-P was greatest at the soil surface and decreased rapidly with depth; this strong stratification, that supports our working hypothesis, can be attributed to total-C stratification considering the correlation between both elements ( $r=0.467$ ,  $p<0.001$ ,  $n=212$ ). As previously reported (Franzluebbers & Hons 1996; Wright et al. 2007), the available-P was more stratified than available-K, likely due to the lower mobility of the former. As for K, the higher P removal by crops during 2008 under RT could be associated with P depletion in deeper layers.

Compared with ryegrass-maize rotations in France and Spain (Lloveras, 1990; Bueno et al. 2007), ryegrass yield was within the normal range but maize yield was poor, probably because of the unfavorable conditions of the unploughed subsoil (>30 cm) for maize rooting. Usually, ryegrass growth do not suffer rainfall deficit in the study area and this was true in 2008. In 2007, emergence and early growth of ryegrass seedlings were probably reduced by the heavy rains during the first month after seeding (264 mm; 15x that in 2008), that strongly leached the fertilizer applied (Couto-Vázquez, personal communication). Contrarily to ryegrass, maize yield can be limited by summer drought in the study area. Although total precipitation during the whole growing period was identical in both years, soil water conditions for emergence and early growth of maize seedlings were likely better in 2008 thanks to higher, but not excessive, precipitations in April-May 2008.

Consistent with other studies on clover and maize from temperate climates (Thierfelder & Wall, 2009; Krauss et al. 2010; Gadermaier et al. 2012; Martínez et al. 2013), we found that conservation tillage did not reduce yield, and even increased it in 2008. The latter result could be related to the dryer

conditions in 2008 because conservation tillage improves soil water conservation and reduces plant water stress (Erenstein, 2002, Thierfelder & Wall, 2009; Krauss et al. 2010). Moreover, García-Marco et al. (2014) findings in a parallel study support this explanation: a) precipitation was much more evenly distributed during maize growth in 2007 than in 2008, which had a drought period in Jun-July, during a critical stage for the young plants growth; and b) compared with CT, the plough layer of RT can store 9.2% more water (495 and 540 Mg/ha, respectively), the importance of this additional water supply in RT (equivalent to 4.5 mm of rainfall) being evident when compared with the 15 mm of rainfall in Jun-July 2008. In 2007, the lack of tillage effect on ryegrass yield may be associated with the abundant precipitation during the early growing season that could lead to high plant mortality and delay in ryegrass development, which in the studied site is more evident under RT (Bueno et al. 2007).

Regardless of tillage system, greater ryegrass nutrient concentrations were obtained in 2007 than in 2008, agreeing with N data in the same plots that were attributed to a smaller ryegrass emergence in the first year and a relative increase in the proportion of tissues of high nutrient content (Couto-Vázquez, personal communication). The significant increase of Mg, Na, K and P contents in ryegrass reflected the positive influence of RT on its nutrition. In contrast, no tillage effects on maize nutrition were observed probably due to the better plant development and hence, larger proportion of structural tissues under RT. As for crop yields, RT had positive effects on plant nutrition during rainfall deficit periods, probably by increasing soil water content that facilitates nutrient uptake. Similarly, De Vita et al. (2007) and Krauss et al. (2010) reported enhanced macronutrient concentration in crops under conservation tillage only in years with low precipitation during the growing season, while no influence was found for wheat and maize in temperate-humid regions (Lavado et al. 2001; Deubel et al. 2011), suggesting that conservation tillage benefits are mainly achieved in dry climates (or dry years).

## Conclusions

Reduced tillage promoted SOM and nutrient accumulation (except K) in the surface layer over the long-term, and it had positive effects on

ryegrass nutrition and, the year with summer drought, on maize yield. Therefore, even in temperate-humid regions, conservation tillage is beneficial for improving water conservation during drought periods, which are predicted to increase in length, severity and frequency due to global warming. Results suggest that there is a need for different fertility management-practices for ryegrass in temperate-humid regions of Spain.

### Acknowledgements

We thank Dr. Alejandra Couto-Vázquez, Dr. Javier Bueno, Jorge Benítez, M<sup>a</sup> Teresa Pérez and Julio Martínez for their valuable help. This research was supported by the Spanish Ministry of Education and Science (project number AGL2005-05726-C02-01) and the post-doctoral JAE-DOC contracts, cofinanced by the European Social Fund, of M.X. Gómez-Rey and S. García-Marco.

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